Investigations towards an efficacious and safe strangles vaccine: submucosal vaccination with a live attenuated *Streptococcus equi*

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As part of a search for a safe and efficacious strangles vaccine, several different vaccines and different vaccination routes were tested in foals. The degree of protection was evaluated after an intranasal challenge with virulent *Streptococcus equi* by clinical, postmortem and bacteriological examinations. Inactivated vaccines containing either native purified M-protein (500 µg per dose) or whole *S equi* cells (10^{10} cells per dose) administered at least twice intramuscularly at intervals of four weeks, did not protect against challenge. Different live attenuated *S equi* mutants administered at least twice at intervals of four weeks by the intranasal route were either safe but not protective or caused strangles. In contrast, a live attenuated deletion mutant administered intramuscularly, induced complete protection but also induced unacceptable local reactions at the site of vaccination. Submucosal vaccination in the inner side of the upper lip with the live attenuated mutant at ≥10^8 colony-forming units per dose, appeared to be safe and efficacious in foals as young as four months of age. The submucosal vaccinations caused small transient swellings that resolved completely within two weeks, and postmortem no vaccine remnants or other abnormalities were found at the site of vaccination.

Streptococcus equi subspecies *equi* causes strangles, a highly contagious disease in the family of Equidae that is characterised by fever and abscess formation in the lymph nodes of the head and the neck. The disease occurs worldwide and causes heavy economic losses in terms of the cost of treatment, quarantine measures and, occasionally, the death of animals.

Most vaccines available on the market have incorporated inactivated whole cells of *S equi* or M-protein extracts. However, such vaccines are notorious for their adverse reactions and induce hardly any protection against natural or experimental infections (Woolcock 1974, Srivastava and Barnum 1981, 1983, 1985, Timoney and Eggers 1985, Sweeney and others 1987, Jorm 1990). Moreover, there is evidence that for protection a mucosal immune response rather than a systemic response is needed (Srivastava and Barnum 1983, 1985, Galan and Timoney 1985, Timoney and Eggers 1985, Timoney and Galan 1985, Galan and others 1986). These studies suggest that the nasopharyngeal mucosal immune system should be triggered by the intranasal administration of an attenuated live vaccine or by purified antigens in a mucosal adjuvant.

As part of a search for a safe and efficacious strangles vaccine the authors have tested several different vaccines and different vaccination routes in horses. A live avirulent deletion mutant administered by the submucosal route (in the inner side of the upper lip) appeared to be the only safe and efficacious method of vaccination.

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MATERIALS AND METHODS

S equi strains

Strain TW is a wildtype *S equi* isolated from a lymph node abscess of a foal with strangles in the Netherlands. This strain was used to prepare the different inactivated vaccines.

Strain TWΔ2Δ3 is a live avirulent deletion mutant derived from *S equi* strain TW (European Patent Application number 786518). Part of a gene essential for the cell's metabolism was deleted. This mutant was constructed by the electroporation of gene knock-out constructs and gene deletion (±1 kb) constructs. In the vaccine mutant strain no vector-derived antibiotic resistance markers or other foreign DNA is present. The mutant strain was tested for haemolysis, capsule synthesis and sugar fermentation, and in all these respects behaved like the wildtype strain.

Strain Arnica is a wildtype *S equi* isolated from a lymph node abscess of a horse with strangles in the Netherlands. This strain was used as the challenge strain and induced strangles in 100 per cent of the control horses tested. Although *S equi* appears to be a clonal pathogen and genetically and immunologically very homogeneous (Galan and Timoney 1988, Jorm and others 1994) a challenge strain different from the vaccine strain was chosen, in order to strengthen the efficacy data.

Horses

For all the experiments Shetland foals ranging in age from four to 16 months with no history of strangles vaccination or disease were used.

Vaccines

Three vaccines were used:

Purified M-protein-based vaccine This vaccine contained 250 µg purified M-protein/ml in purified saponin adjuvant. Each vaccination consisted of 2 ml administered intramuscularly in the neck. The native M-protein was released from the cell wall by the enzymatic incubation of cells of strain TW with lysozyme (10 per cent w/w) and mutanolysin (170 units/g) and subsequently purified in one step by fibrinogen affinity chromatography (Meehan and others 1998). The purified material resolved as one protein band at about 180 kDa in sodium dodecylsulphate (SDS)-polyacrylamide gel electrophoresis, and was stained with Coomassie brilliant blue. Old preparations occasionally resolved at about 58 kDa indicating that the 180 kDa band consists of smaller subunits as described by Meehan and others (1998).

Inactivated whole cell vaccine This vaccine contained $10^{9.7}$ formalin-inactivated cells of strain TW/ml in purified saponin adjuvant. One dose was 2 ml) administered intramuscularly in the neck.

Live avirulent *S equi* strain TW928 vaccine The deletion mutant was freeze-dried in small glass ampoules and reconstituted with distilled water just before use.

For intranasal vaccination (experiment 2) one dose consisted of 2 ml (1 ml into each nostril) containing $10^{8.6}$ colony-forming units (CFU).

For intramuscular vaccination in the neck (experiment 2) one dose consisted of 2 ml containing $10^{8.6}$ CFU.

For submucosal vaccination in the inner side of the upper lip, that is needle injection just below the mucosal layer, one dose consisted of 0·2 ml containing 10^{9} CFU (experiment 3 and 4) or dilutions thereof in physiological saline containing 10^{8} or 10^{7} CFU (experiment 4).

Challenge

In all the experiments the foals (vaccinates and controls) were challenged intranasally two weeks after the last vaccination. One ml of a fresh culture of *S equi* strain Arnica containing about 10^{9} CFU/ml was administered into each nostril with a 2 ml syringe without a needle. This consistently resulted in signs of strangles within five to 10 days in all the control animals tested.

Experiment 1

Six, six-month-old foals were used. Three foals were vaccinated twice intramuscularly, with an interval of four weeks, with the purified M-protein based vaccine, and three foals were left unvaccinated as challenge controls. Two weeks after the second vaccination all six foals were challenged intranasally with *S equi* strain Arnica.

Experiment 2

Twelve yearling horses, 13 to 16 months of age, were used. Three horses were vaccinated three times intranasally at intervals of four weeks with $10^{8.6}$ CFU of the live avirulent *S equi* mutant strain TW928. Three other horses were vaccinated three times intramusculady at intervals of four weeks with the same dose of the same strain. Three other horses were vaccinated three times intramuscularly at intervals of four weeks with 10^{10} cells of the inactivated whole cell vaccine. The last group of three horses was left unvaccinated as challenge controls. Two weeks after the last vaccination all the horses were challenged intranasally with *S equi* strain Arnica.

Experiment 3

Seven foals, nine to 11 months of age were used. Five were vaccinated twice submucosally in the upper lip, with an interval of four weeks, with the deletion mutant strain TW928 and two foals were left unvaccinated as challenge controls. Two weeks after the second vaccination all seven foals were challenged intranasally with *S equi* strain Arnica.

Experiment 4

Sixteen, four-month-old foals were used. In order to determine the minimum protective dose, three groups of four foals were vaccinated twice submucosally in the upper lip, with an interval of four weeks, with deletion mutant strain TW928 at doses of 10^{9} CFU, 10^{8} CFU or 10^{7} CFU. Four horses were left unvaccinated as challenge controls. Two weeks after the second vaccination all the foals were challenged intranasally with *S equi* strain Arnica.

Clinical examination

Just before the challenge, and then at least three times a week, the horses were examined clinically with special attention for signs of strangles. If the horses showed a sudden increase in rectal temperature with clearly swollen submandibular and/or retropharyngeal lymph nodes, whether or not these signs were accompanied by stridor due to obstructed airways, they were regarded as having strangles.

Postmortem examination and bacteriology

In severe cases the horses were killed two weeks after challenge, or otherwise three weeks after challenge, and examined postmortem with special attention to signs of strangles. The diameters (cm) of the abscesses, if present, in the left and right submandibular and retropharyngeal lymph nodes were recorded. Swab samples from various tissues were streaked on to blood agar for bacterial isolation. Swab samples from all the left and right submandibular and retropharyngeal lymph nodes, from all the left and right guttural pouches and from any other abnormal tissues were streaked on to sheep-blood agar for bacteriology. The agar plates were incubated for 18 to 24 hours at 37°C. *S equi* was initially identified by the typical watery β-haemolytic colony morphology and Gram stain and confirmed biochemically by the fermentation of glucose and the lack of fermentation of trehalose, lactose, ribose and sorbitol. *S equi* could be easily distinguished from *S zooepidemicus* because the latter does ferment lactose, ribose and sorbitol.

Enzyme-linked immunosorbent assay (ELISA)

An antibody ELISA against a mutanolysin and lysozyme-solubilised cell wall extract gave high and variable antibody titres, with no differences in titres between vaccinates and controls, most probably because of highly cross-reacting antibodies to *S zooepidemicus*. This opportunistic commensal was isolated from the nasal passages of all the horses in the experiments, in contrast to *S equi* which was only isolated from challenged animals. Before the ELISA was applied the sera were adsorbed with dense suspensions of *S zooepidemicus*. After clearing by centrifugation, serial two-fold dilutions of the adsorbed sera were made in microtitre plates coated with the cell wall extract. After incubation and subsequent washing, bound antibodies were quantified with protein-G conjugate and 3,3'-5,5'tetramethylbenzidine as the substrate. Adsorbing the sera resulted in much lower but more specific *S equi* antibody titres.

RESULTS

Experiment 1: M-protein-based vaccine

Within six days of challenge, all six foals developed clinical signs of strangles characterised by a sudden increase in rectal temperature (>40°C) and swollen lymph nodes in the head and the neck, whether or not accompanied with stridor due to obstructed airways (Table 1). They were about equally affected except for horse 56 which had milder signs. A post-

TABLE 1: Clinical and postmortem results of experiment 1

Horse	Vaccine	Route	Diagnosis	Diameter (cm) of lymph node abscesses postmortem* submn-L	submn-R	retroph-L	retroph-R	Total
55	M-protein	IM	Strangles	3	7	8	10	30
56			Strangles	–	–	3	3	6
59			Strangles	3	4	4	4	15
57	Control		Strangles	7	7	8	8	30
58			Strangles	6	4	7	3	20
60			Strangles	9	5	5	8	27

* From all the abscesses pure cultures of *S equi* were isolated; normal lymph nodes were culture negative

– No abscess present, IM Intramuscular, submn Submandibular, retroph Retropharyngeal, L Left, R Right

TABLE 2: Clinical and postmortem results of experiment 2

Horse	Vaccine CFU/dose	Route	Diagnosis	Diameter (cm) of lymph node abscesses postmortem[a] subm-L	subm-R	retroph-L	retroph-R	Total
35	TW928		Doubtful[b]	–	–	†	†	0
38	$10^{8.0}$	IM	Strangles	5	5	10	10	30
39			Strangles	8	–	–	8	16
34	TW928		Normal	–	–	–	–	0
40	$10^{8.0}$	IM	Normal	–	–	–	–	0
41			Normal	–	–	–	–	0
37	Inactivated		Strangles	10	–	10	–	20
42	whole cell	IM	Strangles	7	7	5	4	23
43	10^{10}		Strangles	–§	–§	–§	1	1
36	Control		Strangles	–	4	5	4	13
45	Control		Strangles	7	10	7	–	24
44	Control		Strangles	6	8	6	6	26

[a] From all the abscesses pure cultures of *S equi* were isolated; normal lymph nodes were culture negative
[b] Intermittent increase in rectal temperature and retropharyngeal lymph nodes sensitive upon palpation
† Enlarged and oedematous but no abscess, *S equi* isolated
§ Very enlarged lymph nodes with no abscess, no *S equi* isolated
– No abscess present, IM Intramuscular, IN Intranasal, CFU Colony-forming units, subm Submandibular, retroph Retropharyngeal, L Left, R Right

TABLE 3: Clinical, postmortem and ELISA results of experiment 3

Horse	Vaccine CFU/dose	Antibody titre[b]	Diagnosis	Diameter (cm) of lymph node abscesses postmortem[a] subm-L	subm-R	retroph-L	retroph-R	Total
2		$2^{3.6}$	Normal	–	–	–	–	0
3	TW928	$2^{3.9}$	Normal	–	–	–	–	0
5	10^9	$2^{4.0}$	Normal	–	–	–	–	0
7		$2^{3.6}$	Normal	–	–	–	–	0
9		$2^{3.0}$	Normal	–	–	–	–	0
1	Control	$2^{3.0}$	Strangles	5	5	4	5	19
8		$2^{3.0}$	Strangles	–	7	–	–	7

[a] From all the abscesses pure cultures of *S equi* were isolated; normal lymph nodes were culture negative
[b] *S equi* antibody titre on day of challenge
– No abscess present, CFU Colony-forming units, subm Submandibular, retroph Retropharyngeal, L Left, R Right

mortem examination two weeks after challenge confirmed the clinical findings. There were large abscesses in the submandibular and retropharyngeal lymph nodes from which pure cultures of *S equi* were isolated.

TABLE 4: Clinical, postmortem and ELISA results of experiment 4

Horse	Vaccine CFU/dose	Antibody titre[b]	Diagnosis	Diameter (cm) of lymph node abscesses postmortem[a] subm-L	subm-R	retroph-L	retroph-R	Total
17		$2^{3.6}$	Normal	–	–	3	–	3
18	TW928	$2^{3.6}$	Normal	–	–	–	–	0
22	10^9	$2^{3.0}$	Normal	–	–	–	–	0
23		$2^{3.0}$	Strangles	5	–	7	7	19
16		$2^{3.6}$	Strangles	–	–	6	8	14
24	TW928	$2^{3.0}$	Normal	–	–	–	–	0
25	10^8	$2^{3.0}$	Normal	–	–	–	–	0
26		$2^{3.0}$	Normal	–	–	3	–	3
21		$2^{3.0}$	Strangles	–	–	6	–	6
27	TW928	$<2^{2.0}$	Strangles	8	9	5	5	25
28	10^7	$<2^{2.0}$	Strangles	8	8	0·5	0·5	17
29		$2^{3.3}$	Strangles	4	1	5	4	14
20		$<2^{2.0}$	Strangles	6	5	4	4	19
30	Control	$2^{3.0}$	Strangles	7	5	7	6	25
31		$2^{3.3}$	Strangles	3	–	5	5	13
32		$2^{3.0}$	Strangles	1	–	6	6	13

[a] From all the abscesses pure cultures of *S equi* were isolated; normal lymph nodes were culture negative
[b] *S equi* antibody titre on day of challenge
– No abscess present, CFU Colony-forming units, subm Submandibular, retroph Retropharyngeal, L Left, R Right

Experiment 2: Inactivated whole cell vaccine versus live TW928

After the intranasal or intramuscular vaccinations no abnormalities were observed except that the three horses vaccinated intramuscularly with deletion mutant strain TW928 developed local reactions at the site of vaccination, that is, local swelling of the neck muscle. After challenge, all three control horses developed severe clinical signs of strangles characterised by high rectal temperatures and swollen and painful lymph nodes of the head and the neck (Table 2). Two of the horses in the group vaccinated intranasally with the live-attenuated mutant (38 and 39), and two of those vaccinated intramuscularly with the inactivated whole cell vaccine (37 and 42) had signs of strangles comparable to those in the controls, whereas the other horses in these two groups (35 and 43) showed milder signs (Table 2). The three horses vaccinated intramuscularly with the deletion mutant were completely protected against strangles; no increase in rectal temperature and no enlarged lymph nodes were observed after challenge. A postmortem examination confirmed the clinical findings. All the horses with clinical signs had abscesses in the submandibular and/or retropharyngeal lymph nodes from which pure cultures of *S equi* were isolated. The three horses that were vaccinated intramuscularly with the deletion mutant appeared to have normal lymph nodes from which *S equi* was not isolated. However, these protected horses had unacceptable local reactions in the form of abscesses at the vaccination site.

Experiment 3: Submucosal vaccination with TW928

After the submucosal vaccinations, small transient reactions were observed at the injection site characterised by small submucosal swellings (2 to 3 cm diameter) which resolved completely within two weeks. The reactions caused no apparent discomfort to the foals which all had a normal appetite.

After challenge, all five foals vaccinated submucosally were protected against strangles whereas both controls developed clear signs of strangles (Table 3). Postmortem examination confirmed the clinical findings. Both control horses had abscesses in the submandibular and/or retropharyngeal lymph nodes from which pure cultures of *S equi* were isolated, whereas all the vaccinated horses had normal lymph nodes from which *S equi* was not isolated. Furthermore, no vaccine remnants or other abnormalities were found at the vaccination sites postmortem. All the vaccinated animals had a *S equi* antibody titre $\geq 2^{3.0}$ whereas both controls had a lower titre.

Experiment 4: Dose-response study with TW928

After the submucosal vaccinations small, transient, dose-dependent submucosal swellings (2 to 3 cm diameter) were observed at the injection site which resolved completely within two weeks. The reactions caused no apparent discomfort to the horses which all had a normal appetite. The group receiving the lowest dose of 10^7 CFU showed no reactions.

Except for three of the horses given 10^9 CFU and three of the horses given 10^8 CFU the horses developed clinical signs of strangles (Table 4). Postmortem examination confirmed the clinical findings, except that horses 17 and 26, although they were clinically protected, had a small abscess (3 cm diameter) in the left retropharyngeal lymph node. However, compared with the controls these horses were clearly less affected. As in experiment 3, the protected animals (except one) had an *S equi* antibody titre $\geq 2^{3.0}$, whereas all the unprotected animals had a lower titre.

Possible correlations between *S equi* antibody titre and protection

In experiments 3 and 4, after submucosal vaccination with the live-attenuated deletion mutant and subsequent challenge, there was an apparent correlation ($r=0.80$) between the

horses' serum antibody titres on the day of challenge and the degree of protection against strangles. All the horses with clinical signs of strangles after challenge had a titre $<2^3$ whereas all but one of the protected horses had titres $\geq 2^3$. A similar correlation ($r=0{\cdot}79$) was found between the antibody titres and the cumulative size of the lymph node abscesses post-mortem. However, more data would be needed to validate these correlations statistically. Furthermore, it is at most an indirect correlation, because after the parenteral vaccinations with the inactivated vaccines in experiments 1 and 2 the horses had titres up to 2^3, but they were not protected.

DISCUSSION

In this study several different vaccines and different vaccination routes were tested in horses. The results show that the combination of live attenuated bacteria and parenteral vaccination is essential for protection against strangles. In particular, live attenuated *S equi* strain TW928 administered by the submucosal route appeared to be a safe and efficacious vaccine.

The M protein belongs to a family of cell surface-associated proteins of streptococci. It is regarded as an important virulence factor (Woolcock 1974, Galan and Timoney 1987, Boschwitz and Timoney 1994, Meehan and others 1998) and therefore most commercially available strangles vaccines for parenteral use are based on either inactivated whole cells or on bacterial extracts, both containing the M-protein. However, according to the literature these vaccines induce hardly any protection against natural or experimental infections (Woolcock 1974, Srivastava and Barnum 1981, 1983, 1985, Timoney and Eggers 1985, Sweeney and others 1987, Jorm 1990). This supposition is supported by the present experiments. In experiment 1, a vaccine containing 500 µg per dose of native purified M protein did not protect horses against strangles after two parenteral vaccinations. However, the same vaccine induced good protection in mice after subcutaneous vaccination and a subsequent lethal intranasal or intraperitoneal challenge (A. Jacobs, unpublished observations) implying that results in mice do not predict the results in horses. Similarly, in experiment 2, a vaccine containing 10^{10} CFU/dose of formalin-inactivated cells did not protect horses against strangles after three parenteral vaccinations. These results indicate that inactivated whole cell or subunit vaccines given by the parenteral route, and possibly a systemic immune response in general, are not protective. In fact the results suggest that a mucosal immune response rather than a systemic immune response may be required for protection. It is also possible that live bacteria grown in vivo have a different antigenic composition than in vitro-grown bacterin antigens. In general, systemic immunity, characterised by a humoral IgG response, is triggered by parenteral (systemic) vaccination, whereas a mucosal immune response, characterised by mucosal IgA, is triggered by presenting antigens to the mucosal surfaces. This can be achieved by the intranasal administration of a live-attenuated vaccine strain or purified antigen combined with a mucosal adjuvant. A live-attenuated deletion mutant, strain TW928, was therefore constructed. Pilot experiments had shown that TW928 was attenuated in mice when tested by the intranasal or intraperitoneal route, and also that it did not cause strangles in foals when using the standard intranasal challenge model (A. Jacobs, unpublished observations). In experiment 2, this mutant was tested as a vaccine by administering it by the intranasal and intramuscular routes. It was surprising that the intranasally vaccinated horses did not appear to be protected whereas the horses vaccinated intramuscularly were completely protected. In contrast with inactivated vaccines given by the parenteral route, a live vaccine given by this route induces protection.

Although TW928 was protective when administered intramuscularly it induced local reactions in the form of abscesses at the site of injection which were regarded as unacceptable for a vaccine to be used in the field. Attempts were therefore made to attenuate this mutant further by constructing additional nitrosoguanidine (NTG)-induced mutations affecting the streptolysin S (SLS) haemolysin and the bacterial capsule, resulting in double or triple mutants. Single or double mutants defective in SLS haemolysin and the capsule but lacking the original attenuating lesion were also prepared. However these mutants, when tested by the intramuscular route were either safe but not protective, or protective but not safe, as indicated by local reactions at the vaccination site (A. Jacobs, unpublished observations). Similarly, when they were tested by the intranasal route, the mutants were either safe but not protective, or actually caused strangles. An SLS (haemolysin)-negative mutant and an SLS/capsule double mutant derived from *S equi* strain TW, although they were both strongly attenuated in mice, caused strangles in yearling horses, with the mutant strains being isolated from the lymph node abscesses (A. Jacobs, unpublished observations). Apparently *S equi* can cause strangles without the SLS haemolysin and/or capsule. This result is consistent with the results of Galan and others (1988) who found that a capsule-defective mutant of *S equi* still caused strangles in young foals. Since these trials showed that further attenuation did not improve either the safety or the efficacy of the vaccine when administered by the intramuscular or intranasal routes, another vaccination site was explored. In experiment 3, the horses were vaccinated submucosally in the inner side of the upper lip just below the mucosal layer. This new parenteral vaccination route appeared to be safe as well as efficacious. Only transient small submucosal swellings were observed which resolved completely within two weeks, and no residues or other abnormalities were found at the injection site post-mortem. Furthermore, all five vaccinated horses appeared to be protected, in contrast with the two challenge controls which both developed strangles. In experiment 4 the minimum protective dose was established at 10^8 CFU. It can be concluded that strain TW928 is a promising candidate vaccine. Furthermore, the fact that it is a deletion mutant makes it highly unlikely that it can revert to virulence.

The intriguing question of the mechanism of protection remains to be answered. There is accumulating evidence that a mucosal immune response is essential for protection against strangles (Srivastava and Barnum 1983, 1985, Galan and Timoney 1985, Timoney and Eggers 1985, Timoney and Galan 1985, Galan and others 1986). However, the present results do not confirm this hypothesis because the live-attenuated vaccines tested intranasally were either safe but not protective or caused strangles. This indicates that the optimal attenuation for the intranasal route is difficult to reach or does not exist at all. In contrast, the results show that systemic vaccination induces good protection provided that a live vaccine is used. The fact that the vaccine was delivered by the parenteral route, and the apparent correlation between the antibody titres and the level of protection both suggest that the protection might be due to a systemic immune response. On the other hand, this only works when a live vaccine is used because the inactivated whole cell vaccine and the M-protein-based subunit vaccine afforded no protection. In addition, the apparent correlation between the antibody titres and protection was only observed when a live vaccine was used via the parenteral route. After parenteral vaccination with the inactivated whole cell vaccine or the M-protein-based subunit vaccine, antibody titres $>2^3$ were observed, but the horses were not protected. These discrepancies might be explained by the upregulation of additional antigens (essential for inducing protection) in the live vaccine strain in vivo. Furthermore, live bacteria could trigger a different and/or

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THE NEWSLETTER FOR BENEFACTORS OF GRAYSON-JOCKEY CLUB RESEARCH FOUNDATION, INC.

SEEKING SOLUTION TO STRANGLES

(Editor's Note: The march of progress in medicine is often a long and difficult one, marked more by frustrations than by chances to shout "eureka!" Determination perhaps is no less important to a scientist as knowledge and intellectual inquiry. Among researchers who have been funded by Grayson-Jockey Club Research Foundation is Dr. John Timoney of the University of Kentucky, as he works toward a safe, reliable vaccination for a painful equine disease. The following illustrates both the difficulties, and importance, of such journeys.)

Research involving the causative bacterium of the disease known to horsemen as "strangles" has been an ongoing interest of Dr. John Timoney for many years. Dr. Timoney has been responsible for much of the seminal work involving Streptococcus equi, and is currently delving specifically into the search for new components of the organism's structure or molecular composition which may function as immunogens, or units which alert the horse's immune system and elicit a containment response towards the pathogen. Despite the last decade's advances and forward strides in our understanding of S. equi and its manifestations of infection, many feel that a vaccination preparation which is reliable with regard to both safety and efficacy has yet to be developed. Timoney's present investigation focuses on immunogenic portions of the bacterium which are present in addition to M protein, the current component of parenteral strangles vaccines.

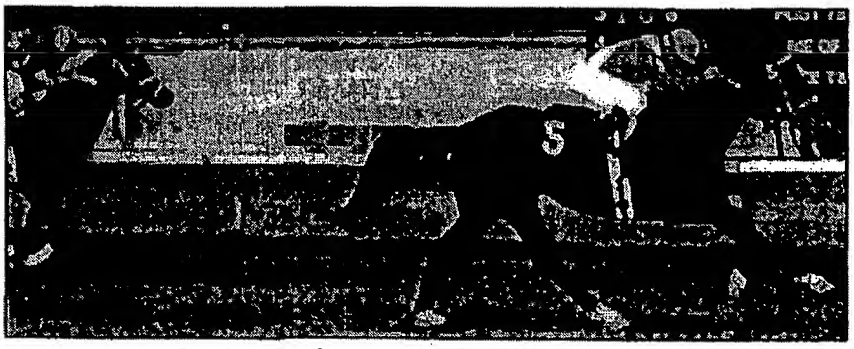
As information is developed about these additional protein immunogens, it is likely to lead to an improved vaccine preparation, eliciting a containment response toward the pathogen that more closely parallels what occurs in natural infections. The importance of this work lies in the possibility that a substantial improvement in our ability to successfully immunize horses against strangles will result.

In its crudest form, vaccination is achieved by simply injecting an emulsion or suspension of a pathogen into an animal. The dose of pathogen injected must be small enough to avoid overwhelming the immune system and causing the very clinical signs of disease the vaccination process is trying to prevent, yet must be sizeable enough to be "perceived" by the immune system *(continued on page 2)*

Races Named for Grayson-Jockey Club

Several race tracks have provided opportunities for the Foundation to achieve increased visibility by naming overnight races for Grayson-Jockey Club. The first Grayson-Jockey Club Purse was held at little Rillito in Tucson, AZ, on Feb. 17, and was won by Jose A. Barrios' Cop Out. More recently, Prairie Meadows held its Grayson-Jockey Club Purse on May 24. B. E. Howerter's Bonita Rose won the $25,000 event. Dr. Scott McClure, whose project on shock wave therapy at Iowa State University is being funded by Grayson-Jockey Club, was interviewed before and after the event on Prairie Meadows' in-house television system.

As of press time, Suffolk Downs, Calder, Belmont Park, and Emerald Downs also were planning races named for the Foundation. Grayson-Jockey Club appreciates the opportunities to explain to fans and horsemen alike the functions of the Foundation and how any individual can participate.



Cop Out wins the Grayson-Jockey Club race at Rillito.

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REMEMBERING GENEROUS LEADERS

In recent months, Grayson-Jockey Club Research Foundation, and all of the horse world, lost two of its staunch supporters through the deaths of Ogden Phipps and Mrs. Alice Mills. Mr. Phipps and his family have been longtime supporters of the Foundation through leadership as well as generosity.

Recent contributions included a portion of a stallion season in Seeking the Gold auctioned at Keeneland. The large number of memorial contributions made to the Foundation in Mr. Phipps' memory is testimony to the respect he engendered on the Turf.

Mrs. Mills, who was director emeritus of the Foundation at the time of her death, also had been a supporter. She and her late husband, James P. Mills, made a major contribution to the Foundation in 1985 from the earnings of their champion Devil's Bag.

(continued from page 1) and elicit the appropriate protective response.

Vaccination of horses against the bacterium Streptococcus equi has traditionally been plagued by complications, most notably the development of reactions or abscesses at the injection site, and also by the development of the sometimes-fatal autoimmune disease "purpura hemorrhagica." Purpura also accompanies naturally-acquired infection. Some horses actually contracted strangles from their vaccine, while conversely, in other situations there was a documented failure of immunization to protect the animal when challenged by natural exposure. Given these problems, equine veterinary practitioners tend to recommend implementation of vaccination programs for strangles only when the horse in question resides on an endemic property or will be traveling to a high-risk exposure situation.

In the 1980s, the safety aspect of vaccinating for strangles was enhanced by the development of sub-unit vaccines. Research identified a specific region of the bacterial cell wall, protein SeM, as being a highly immunogenic portion of the bacterium and able to induce as protective an immune response as that induced by a suspension of the entire bacterium. Production of these vaccines involved preparing suspensions of bacterial fragments containing only the bacterial cell wall components; administration of such products to horses was safer because vaccination could not lead to the development of active infection. Still, purpura hemorrhagica and sterile abscessation at the injection site continued to be specters which attended vaccination of horses for strangles. The fact that vaccinations made from protein M are given parenterally (intramuscularly) also means that the efficacy of this procedure could be less than entirely reliable, because while circulating antibodies were made by the immune system in response, antibodies at the portal of entry were not elicited.

Most recently, researchers and commercial vaccine producers have focused upon targeting pathogens at their portals of entry, where infection first begins. For bacteria like S. equi, this means inducing protective antibody production in the tissue lining (the mucosa) of the upper respiratory tract.

Currently, intra-nasal vaccination for strangles is available, but this mode of immunization, like its predecessors, has experienced a troublesome relationship between efficacy and safety. The vaccine preparation currently available is a suspension of Strep. equi bacteria which are live, but attenuated so that they have reduced virulence.

While many horses have no adverse complications and appear to be protected, some horses which received their intra-nasal strangles vaccination at the same time that they were given their intramuscular immunizations (such as 4-way, rhinopneumonitis, influenza) developed strangles abscesses at the site of injection of the other vaccines. This unique complication arose from live bacteria in the vaccine getting on the hands of the person administering the vaccinations and *(continued on page 3)*

A fund raising trail ride and tour of historic Groton Plantation in Aiken, SC, was held during the spring. Here, riders are seen in front of Oakland Hall on the property. The ride was hosted by Mike and Iris Freeman. In the West, another trail ride as a Grayson-Jockey Club fund raiser was held on the T-4 Ranch of Forrest, Kim, and Jenifer Metz in Patagonia, AZ.

(continued from page 2) contaminating the needles and syringes used to administer the intramuscular doses. Apparent failure of vaccination to protect against clinical disease in certain cases, the occasional occurrence of purpura hemorrhagica, and even the development of strangles from the vaccine have all been documented in association with intranasal vaccination.

Streptococcal bacteria are responsible for serious disease in human beings, too. Streptococcal pharyngitis ("strep throat"), scarlet fever, toxic shock syndrome, and necrotizing fasciitis, a frightening disease which consumes living tissue and is associated with high mortality, are all caused by streptococcal organisms. Of the genus Streptococcus, S. zooepidemicus is the species of bacterium which has evolved to co-exist with the horse. It is the most frequently cultured organism from a variety of equine infections.

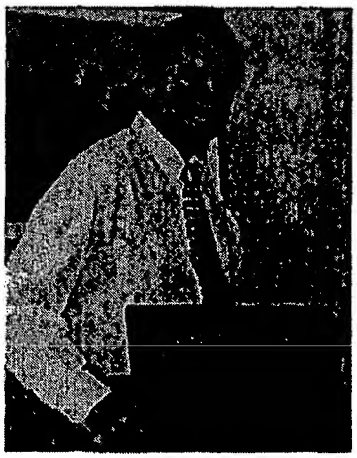
Research in Dr. Timoney's lab at the Gluck Equine Research Center has resulted in the development of a number of important advancements in our knowledge about Streptococcus equi. That S. equi is a more virulent clone of an ancestral S. zooepidemicus has become apparent from the over 97% commonality of DNA that Strep. zoo and Strep. equi share. The 2-3% of the genetic material that is not shared with Strep. zoo and that is unique to Strep. equi is the focus of Timoney's current work. His present research investigates the hypothesis that it is this unique portion of the bacterium's genome which will code for immunogenic proteins which are specifically protective against strangles and which, when added to existing vaccine suspensions, may significantly improve efficacy. Timoney specifies that an antibody response on two different levels is needed in order for a vaccinated horse to be protected. First, antibodies produced at the surface of the mucous membrane where organisms first invade are necessary, because the presence of antibodies there will bind and neutralize the infectious organisms, preventing their binding to the horse's tissues. Second, antibodies must additionally be present in the tissues which lie between that pharyngeal lining and the deeper lymph nodes, where they perform the same function, namely to bind and neutralize bacteria so that metastasis of infection to deeper lymph nodes ("bastard strangles") is prevented. Dr. Timoney feels that if a nasal vaccine can successfully prompt the above type of immune response, it would be conceivably possible to eradicate strangles from a vaccinated, closed herd. This has great implications for owners of farms or premises upon which the infection has become endemic, with cycles of outbreaks of clinical disease. The costs of quarantining affected horses, lost business, veterinary treatment, and the time of farm personnel in monitoring and caring for ill horses are considerable, and may in some circumstances be devastating.

Despite the inherent frustrations and difficulties associated with immunizing horses against S. equi, Dr. Timoney feels like successful vaccination is nevertheless a goal with an end in sight: "It certainly is possible that effective protection will be available to horses in the foreseeable future. Horses which have had strangles are quite resistant to a second infection, so nature herself tells us that effective 'vaccination' is possible."

The surface M protein (SeM), which has formed the basis of the parenterally administered subunit vaccines, is highly immunogenic and will probably remain a primary component of future vaccine preparations. Timoney's work has identified two additional proteins on the bacterium's surface, SePE-H, and SePE-I, which may appear to be key players in eliciting an immune response from the horse. All three of these immunogenic surface proteins, SeM, SePE-H and SePE-I, were first recognized and characterized in Timoney's lab. The Grayson-Jockey *(continued on page 4)*

Dubai Millennium Memorial Research Award

Dr. Philip Johnson recently received the Grayson-Jockey Club Research Foundation's Dubai Millennium Memorial Research Award. Dr. Johnson is conducting a project on laminitis at the University of Missouri. The award is named for a deceased champion which raced for the Maktoum family's Godolphin stable. A season in Dubai Millennium was donated by Sheikh Mohammed for auction on behalf of the Foundation late in 2000. The entire proceeds of the sale, $270,000, were donated to the Foundation, which instituted the award in appreciation.



(continued from page 3) Club Research Foundation has been a long-term supporter of Dr. Timoney's work, and, as such, has had a direct role in the development of much of what comprises current thought about Streptococcus equi.

Dr. Timoney credits the Sanger Sequencing Center at the University of Cambridge for defining the genomic sequence of S. equi and then providing unrestricted access to the information for the improvement of equine health. S. equi is the first equine bacterial pathogen to be sequenced in its entirety. The project to elucidate the gene sequence for this organism was underwritten by the Home for Rest for Horses. The project's support at determining the organism's gene sequence together with the policy of free access generously provided to all investigators significantly accelerates the ability of researchers to identify new immunogens for possible inclusion into new-generation vaccine preparations. Dr. Timoney's lab, so instrumental in developing information about S. equi in the past decade, is uniquely positioned to make use of the knowledge contained in the bacterium's gene sequence.

The genes which code for the three unique wall proteins of S. equi are part of the 2-3% difference in genetic material that exists between S. equi and S. zooepidemicus. Dr. Timoney stipulates that there are probably a total of 20 to 30 proteins included in this sequence, however, and that additional proteins are likely present which will turn out to be important in the horse's immunogenic response to strangles. Such bacterial proteins which elicit a significant immune response would be targeted for investigation into their possible suitability for inclusion in future vaccines. Dr. Timoney postulates, for example, that SePE-I may be one of the bacterial cell wall proteins which stimulates an immune response in the infected horse. Antibodies made against SePE-I may be what confers the resistance to future infections seen in horses naturally infected with the strangles organism. If so, then SePE-I could be an important addition to protein M (SeM) in future vaccines.

– By Kim A. Sprayberry, DVM, Diplomate ACVIM

The grant supporting Dr. Timoney's 2001-2002 work is entitled "Identification of immunogenic proteins unique to Streptococcus equi." The Grayson-Jockey Club Research Foundation is proud to be a long-time supporter of Dr. Timoney's research and to fund such investigations whose outcome is anticipated to impact equine health so positively.

NEW YORK THOROUGHBRED HORSEMEN SUPPORT EQUINE RESEARCH

For the second time in two years, the New York Thoroughbred Horsemen's Association has made a major contribution to Grayson-Jockey Club Research Foundation. The NYTHA recently donated $37,000 to the Foundation, which is a leader in sponsoring research dedicated to improving the health and safety of horses.

A portion of the contribution was designated as in memory of Ogden Phipps, a patriarchal sportsman of the Turf who died recently after a long and distinguished career as an owner-breeder and leader in racing.

"We at the NYTHA appreciate that Grayson-Jockey Club seeks out and funds the best research available on the most important problems facing the horse," said the organization's executive director, Robert F. Flynn. "When mares started losing foals in large numbers last year, the Foundation stepped up immediately to fund several projects seeking answers. At the same time, that did not diminish its commitment to seeking solutions to other problems all horses and horse owners face."

Grayson-Jockey Club currently is funding 24 projects for more than $800,000 and over the last two decades has supported 180 projects at 31 universities for a total of more than $0.5 million.

Problems addressed include various infectious diseases, laminitis, and an array of soundness issues. One of the projects underway seeks to develop a means of alerting horsemen to impending injury of bone and joint through analysis of serum markers. Several projects also utilize cutting edge technology such as use of adult stem cells to aid cartilage regeneration.

Our Foundation is dependent on the generosity of the horse community. We are in business solely to help the horse, and when organizations such as the NYTHA come forward so generously, it is an important boost to all who are connected to the equine industry.

Rokeby Circle Members

In honor of the generosity to the Foundation by the late Paul Mellon, Grayson-Jockey Club designates as members of the Rokeby Circle those donors/members at the $10,000-plus level in a given year. The honor is named for Rokeby Farm, Mr. Mellon's beloved estate in Virginia. Current members of the Rokeby Circle as of June 1:

Abercrombie Foundation
Mr. & Mrs. Joseph Allen
Ashford Stud
William Backer
Gary Biszantz (Cobra Farm)
Lucy Young Boutin
Bronzino Knight Trust
Brookdale Farm
Alex G. Campbell
Churchill Downs
Claiborne Farm
Mr. & Mrs. Dennis Dammerman
Darley Stud
Del Mar Thoroughbred Club
Adele B. Dilschneider
Donald Dizney
The Jane & Allan Dragone Foundation
Richard L. Duchossois
Mrs. Allaire duPont
R. S. Evans Foundation
The William Stamps Farish Fund
Tracy Farmer Foundation
John K. Goodman
Seth W. Hancock
Hellinger Foundation
R. D. & Joan Dale Hubbard Foundation
G. Watts Humphrey Jr.
Stuart S. Janney III
Keeneland Association
Keeneland Foundation
Landon Family Foundation
Lane's End Farm
Lavin Bloodstock Services
Robert & Beverly Lewis
Mr. & Mrs. Eugene Melnyk
Robert E. Meyerhoff
Leverett S. Miller
Mockingbird Farm (Barry T. Mangurian Jr.)
National City Bank
New York Racing Association
New York Thoroughbred Horsemen's Assoc
Northern Trust
Oak Tree Racing Association
John M. B. O'Connor
Mr. & Mrs. Paul Oreffice
Overbrook Farm (William T. Young)
Mr. & Mrs. John Oxley
Emie Paragallo
Ogden M. Phipps
Carl Pollard
Richard Santulli
Mr. & Mrs. Paul Schosberg
Joseph V. Shields Jr.
Stonerside Stable LLC
Dwight Sutherland
Taylor Made Farm
Thoroughbred Charities of America
Thoroughbred Corporation
Kenny & Lisa Troutt
Windfields Farm
XL Specialty

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Wyeth Red
PMS 107
PMS 355

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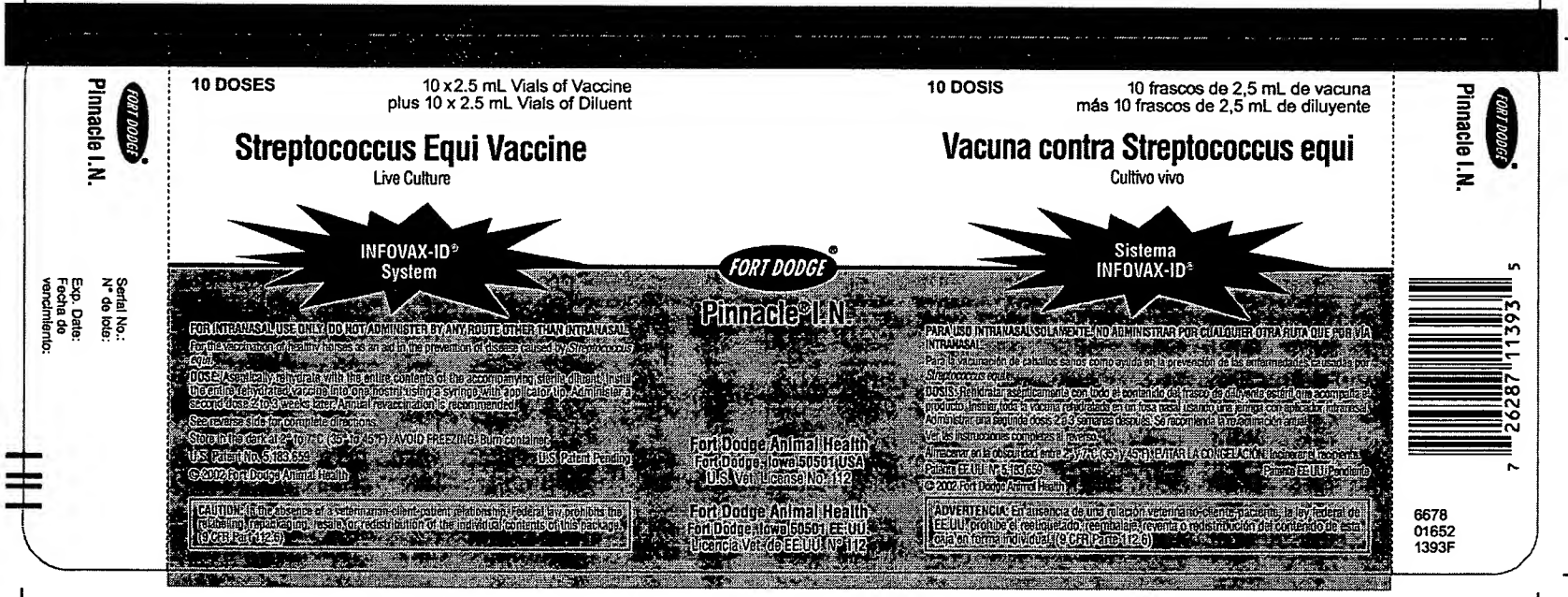


PMS 287
PMS 277

TOP

FOR INTRANASAL USE ONLY. DO NOT ADMINISTER BY ANY ROUTE OTHER THAN INTRANASAL.

For the vaccination of healthy horses as an aid in the prevention of disease caused by *Streptococcus equi*.

DOSE: Aseptically rehydrate with the entire contents of the accompanying sterile diluent. Instill the entire rehydrated vaccine into one nostril using a syringe with applicator tip. Administer a second dose 2 to 3 weeks later. Annual revaccination is recommended.

CAUTION: This product contains live bacteria and is designed for intranasal use only. Disinfect hands and equipment after use. Contamination of the user's hands or equipment with reconstituted vaccine could lead to infections if proper disinfection practices are not followed prior to procedures that require asepsis. Injection equipment used to reconstitute or administer Pinnacle I.N. should not be reused, and should be disposed of appropriately. In case of anaphylactoid reaction, administer ephephrine. After administration a small number of horses may experience non-contagious transitory upper respiratory signs including nasal discharge and lymphadenectasis. Purpura hemorrhagica may be seen in hypersensitive individuals following exposure to streptococcal proteins. Store in the dark at 2° to 7°C (35° to 45°F). AVOID FREEZING. Shake well after rehydration. Do not vaccinate within 30 days before slaughter. Use entire contents when first opened. Burn container and all unused contents.

U.S. Patent No. 5,183,659 U.S. Patent Pending

For Veterinary Use Only

1393F

INFOVAX-ID® System

The INFOVAX-ID System provides a simple and effective method of recording pertinent information on the vaccines administered to animals in a veterinary practice.

For vaccines requiring reconstitution, remove label from both vials and affix both labels to the animal's medical chart.

Using the INFOVAX-ID System:

U.S. Pat. No. 5,704,648

1. Grasp the lower right hand corner of the tab at the arrow marked "Peel Here" between your thumb and forefinger. (figure 1)


(fig. 1)

2. Pull steadily at a slight upward angle until the top portion of the label is separated from the vial. (figure 2)


(fig. 2)

3. Place the label on the animal's medical chart.
4. Press down on the label to ensure adhesion.

Sistema INFOVAX-ID®

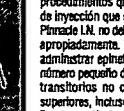
El sistema INFOVAX-ID proporciona un método sencillo y efectivo de registrar la información pertinente sobre las vacunas administradas a los animales en la práctica veterinaria.

Para las vacunas que requieren reconstitución, quitar la etiqueta de ambos frascos y fijarlas al registro médico del animal.

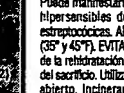
Uso del sistema INFOVAX-ID:

Pat. de EE.UU. N° 5,704,648

1. Asir entre pulgar e índice la esquina inferior derecha de la etiqueta en la flecha marcada "Despegue aquí". (figura 1)


(fig. 1)

2. Despender tirando firmemente y a un ángulo un tanto ascendente, hasta que la parte superior de la etiqueta quede separada del frasco (figura 2)


(fig. 2)

3. Colocar la etiqueta en el registro médico del animal. Presionar en la etiqueta para asegurar su adhesión.

PARA USO INTRANASAL SOLAMENTE. NO ADMINISTRAR POR CUALQUIER OTRA RUTA QUE POR VÍA INTRANASAL.

Para la vacunación de caballos sanos como ayuda en la prevención de las enfermedades causadas por *Streptococcus equi*.

DOSIS: Rehidratar asépticamente con todo el contenido del frasco de diluyente estéril que acompaña el producto. Instilar toda la vacuna rehidratada en un fosa nasal usando una jeringa con aplicador intranasal. Administrar una segunda dosis 2 a 3 semanas después. Se recomienda la revacunación anual

PRECAUCIÓN: Este producto contiene bacterias vivas y está diseñado exclusivamente para uso intranasal. Desinfectar las manos e instrumentos después del uso. La contaminación de las manos del usuario o de los instrumentos con la vacuna reconstituida puede causar infecciones si no se siguen apropiadas prácticas de desinfección antes de realizar procedimientos que requieran asepsis. Los instrumentos de inyección que se usen para reconstituir o administrar Pinnacle I.N. no deben usarse otra vez y deberán desecharse apropiadamente. En caso de una reacción anafilactoida, administrar epinefrina. Después de la administración, un número pequeño de caballos puede experimentar síntomas transitorios no contagiosos de las vías respiratorias superiores, incluso secreciones nasales y linfadenectasia. Puede manifestarse Purpura hemorrhagica en individuos hipersensibles después de la exposición a proteínas estreptocócicas. Almacenar en la obscuridad entre 2° y 7°C (35° y 45°F). EVITAR LA CONGELACIÓN. Agitar bien después de la rehidratación. No vacunar dentro de los 30 días antes del sacrificio. Utilizar todo el contenido de este frasco una vez abierto. Incinerar el recipiente y todo el contenido no utilizado.

Patente EE.UU. N° 5,183,659 Patente EE.UU. Pendiente

Uso veterinario

Biosecurity and Health Committee Protocol for the Management of Strangles in Racehorses

Author: Biosecurity and Health Committee: Canadian Pari-Mutual Agency; The Horsemen's Benevolent and Protective Association of Ontario; Ontario Harness Horse Association; Ontario Horse Racing Industry Association; Ontario Ministry of Agriculture and Food; Ontario Racing Commission; University of Guelph; Woodbine Entertainment Group.

Creation Date: 01 September 2003
Last Reviewed: 01 September 2003

Table of Contents

Section 1: Disease Information

Strangles is a highly contagious and serious infection of horses and other equids caused by the bacterium *Streptococcus equi (S. equi)*. The disease is characterized by severe inflammation of the mucosa of the head and throat, with extensive swelling and often rupture of the lymph nodes, which produces large amounts of thick, creamy pus.

Section 2: Human Health Risk Data

Humans appear to be resistant to *S. equi* under normal circumstances.

Section 3: Horse Health Risk Data

Horses of all ages are susceptible, though strangles is most common in animals less than five years of age and especially in groups of weanling foals or yearlings. Animals show typical signs of a generalized infectious process (depression, inappetence, fever of 39° - 39.5°C). Horses develop a nasal discharge (initially mucoid, rapidly thickening and purulent), a soft cough and slight but painful swelling between the mandibles, with swelling of the submandibular lymph node. With the progression of the disease, abscesses develop in the submandibular (between the jaw bones) and/or retropharyngeal (at the back of the throat) lymph nodes. The lymph nodes become hard and very painful, and may obstruct breathing ("strangles"). The lymph node abscesses will burst (or can be lanced) in 7 to 14 days, releasing thick pus heavily contaminated with *S. equi*. The horse will usually rapidly recover once abscesses have ruptured.

Section 4: Ecology Information

S. equi is maintained in the horse population by carrier horses but does not survive for more than six to eight weeks in the environment. The infection is highly contagious. Transmission is either by direct or indirect contact of susceptible animals with a diseased horse. The incubation period of strangles is usually 3 to 14 days. Direct contact includes contact with a horse that is incubating strangles or has just recovered from the infection, or with an apparently clinically unaffected long-term carrier. Indirect contact occurs when an animal comes in contact with a contaminated stable (buckets, feed, walls, doors) or pasture environment (grass, fences, but almost always the water troughs), or through flies. Under optimal conditions, the bacteria can survive probably six to eight weeks in the environment.

Section 5: Prevention

Both a killed and a live vaccine are available for the control of strangles. The only killed vaccine currently available in Canada is Strepguard™ by Intervet. Killed vaccines, in general, are administered with an initial series of intramuscular injections followed by an annual booster. There may be adverse reactions at the injection site (marked pain, even frank abscesses). Some animals have even developed purpura haemorrhagica following vaccination. The killed vaccines do not provide complete protection because they do not result in the local, nasopharyngeal antibodies thought to be important in protection, but they may reduce the severity of clinical illness should it occur.

More recently, a live, attenuated *S. equi* vaccine (Pinnacle™ I.N. by Fort Dodge) has been introduced as an intranasal vaccine for the prevention of strangles. The vaccine is administered twice, at an interval of one to two weeks. This approach to vaccination is intuitively more attractive than a killed, intramuscular vaccine since it produces the local antibodies necessary for protective immunity. Because the vaccine is a live but attenuated (using a low virulence organism) *S. equi*, care should be taken to avoid contamination of injections elsewhere in the horse. Concurrent injection of other vaccines has resulted in *S. equi* abscesses at these sites, presumably through inadvertent contamination.

Jorm (1991) has shown that *S. equi* survived for 63 days on wood at 2°C and for 48 days on glass or wood at 20°C. The organism is readily killed by heat (60°C) or disinfectants (particularly povidone iodine, chlorhexidine). Quarantine area staff should change their coveralls and boots before leaving the quarantine area, and should wash their arms and hands carefully with chlorhexidine soap or use an alcohol-based hand disinfectant solution.

Infected horses should be isolated and not allowed to come into contact with other horses until they are no longer shedding *S. equi*. Personnel working with infected horses should not work with other horses, or should work with infected horses last. Clothing should be changed after working with an infected horse, and hands should be thoroughly washed. Any items coming in contact with an infected horse or its stall (hay nets, water buckets, etc.) should be disinfected before being used for another horse. Infected horses can shed *S. equi* for weeks. Contaminated pasture areas should be rested for four weeks, since the organism will be killed by the natural antibacterial effects of drying and of ultraviolet light. Once a case of strangles has been identified, all horses that have been in contact with the affected horse should be considered potentially exposed. Their body temperature should be monitored closely to detect infection as early as possible. Ideally, horses should not leave the premises after an infected horse has been identified, unless they have been tested and determined not to be carrying *S. equi*.

New arrivals to a barn should be quarantined for at least 2 (and ideally 3) weeks. All quarantined horses should be considered a potential source of *S. equi*, even if they appear healthy. Depending on the situation, screening for *S. equi* might be recommended. This would consist of testing for the presence of *S. equi* in the nasopharynx (nose and throat region) and guttural pouches.

Section 6: Regulatory Information

Strangles is not a reportable disease and, therefore, outbreaks of this disease are not required to be reported to any government agency.

Committee's Recommendations

1. All "pony" horses shall have completed their vaccination program (initial and booster shots) for strangles at least two weeks prior to arrival at the track.

2. It is recommended that all racehorses be vaccinated with the intranasal vaccine for strangles (initial and booster shots) prior to arrival at the track.
3. Track owners should install wash stations with hand disinfectant at strategic locations along each shed row or barn for personal hygiene when working between horses.
4. All personnel should wash their hands after working with each horse under their care.
5. High pressure washers and supplies should be available at the track to disinfect stalls and equipment. However, dirt floor stalls with wood walls will require removal of infected dirt (upper 2") and scrubbing of the walls.
6. Horses purchased at sales should be quarantined for 2 - 3 weeks prior to having contact with other horses.
7. Horses from farms with cases of strangles on the property should not be admitted to a racetrack until they have undergone a 2-3-week quarantine.

More Information

Strangles in Horses, Ontario Ministry of Agriculture and Food - http://www.gov.on.ca/OMAFRA/english/livestock/horses/facts/03-037.htm

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For more information:
Toll Free: 1-877-424-1300
Local: (519) 826-4047
Email: ag.info@omaf.gov.on.ca

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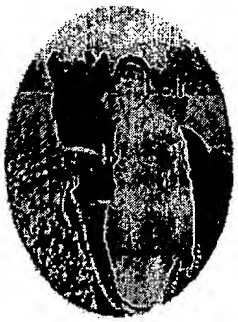
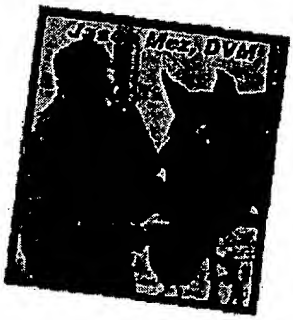


Ramblings...

An Open Horse Clinic featuring wild horses was sponsored by South Dakota's **Wildhorse Club.calm** on March 22, 2003. The event was held at the Black Hills Equestrian Center in Rapid City, SD. Clinicians covered healthcare, showmanship, English riding, cowhorse training, trick training, roundpen work, and western pleasure. Adopted wild horses were used for the training demonstrations, which goes to show that these horses are versatile.

Below are some photos of the event, along with some points I took note of:

Jason Mez, DVM - Healthcare



Dr. Mez had a plethora of information to impart regarding vaccination schedules, de-worming schedules, nutrition, dental care, and hoof care.

Vaccination schedules - Performance horses should be vaccinated for Eastern/Western Encephalomyelitis (sleeping sickness), Tetanus, Rhino, Flu, Rabies, and West Nile Virus every spring. Horses that are hauled and used extensively, such as show circuit horses or those involved in PRCA, should also be vaccinated in the fall for Rhino and Flu.

Broodmares need a modified live Rhino vaccine in the 5^{th} , 7^{th} , and 9^{th} month of pregnancy. They should receive sleeping sickness, Tetanus, Flu, Rhino, and West Nile Virus 4-6 weeks prior to foaling. If you give rabies vaccine, this should be given prior to breeding. A Rhino booster should also be given at breeding time.

Foals from vaccinated mares should receive sleeping sickness, Tetanus, Flu, Rhino, and West Nile Virus at 6 months with 2 additional boosters given at 7 and 8 months. Rabies should be given at 6 months with a booster at one year.

Foals from non-vaccinated mares should receive sleeping sickness, Tetanus, Flu, Rhino, and West Nile Virus at 3 months with 2 additional boosters at 4 and 5 months. Rabies should be given at 3 months with a booster at one year.

At a minimum, all horses should be vaccinated for Eastern/Western Encephalomyelitis, Tetanus, and West Nile Virus in the spring every year.

Dr. Mez does not recommend the Strangles vaccination. He says there is very strong evidence that vaccinated horses exposed to Strangles have a high incidence of Purpurae Hemorhagica, which is a debilitating and often times fatal condition best characterized as an autoimmune condition. However, if you want to vaccinate for Strangles, the intranasal

product is superior to the intramuscular vaccine.

Rabies is optional but highly recommended as there are cases every year in this area.

Dr. Mez recommends the intranasal flu vaccine over the intramuscular vaccine. NOTE: If using both the intranasal flu and strangles vaccines, they must be given one week apart.

West Nile Virus requires 2 shots three weeks apart, initially. Then a yearly booster is needed in the spring. Research has shown that the vaccine is effective up to 13 months.

De-Worming schedule - Dr. Mez recommends de-worming every three months. De-wormers should be rotated throughout the year. Never use an Avermectin product the first time you worm a foal or a horse that has never been de-wormed before.

Month	Wormer
Jan-Feb-Mar	Benzimidazole
Apr-May-June	Benzimidazole
July-Aug-Sept	Pyrantel
Oct-Nov-Dec	Avermectin

- **Avermectins: Eqvalan, Zimecterin, Equimectrim, Quest**
- **Benzimidazoles: Panacur, Safe-guard, Anthelcide**
- **Pyrantel: Strongid-P, Strongid-T, Strongid-C, Rotectin-2**

Broodmares should be de-wormed a minimum of 2 times per year, and Panacur should be used just prior to foaling. Avermectin should be used in the fall after a hard frost. De-worm foals at 30 days of age with a double dose of Panacur, then again at weaning time.

Nutrition - Horses should have free access to clean, fresh water. Keep the water free of ice during the winter. A horse will drink 10-15 gallons of water per day during the winter and as much as 30 gallons a day during the summer. The best feed for horses is pasture. Alfalfa is NOT bad for a horse, unless the horse has an existing kidney problem. Always have salt with trace minerals available. Salt blocks are acceptable.

Dental care - Horses of all ages may require dental care. Young horses (2-5 years) typically have the most dental problems because they are losing teeth and growing new teeth. Feeding horses on the ground or in a low trough results in less dental problems than feeding in a raised trough (this has something to do with the angles of feeding and chewing). Sweet feeds (grain with molasses) are not bad for a horse's teeth. If you think

your horse has sharp points on his teeth, feel the teeth on the outside of the mouth, not the inside (good way to get bit!). If the horse tosses his head and generally acts like he doesn't like you rubbing along his molars, he probably has problems. Dr. Mez recommends that horses have their teeth checked annually if stalled; pastured horses tend to not have dental problems.

Hoof care - Studies in Europe are showing that NOT shoeing is better for a horse.

Final comments by Dr. Mez - horse owners need to think in advance how they want to handle problems such as colic and serious joint injuries. Treatment of these problems can become very expensive, and results may not be optimal. It's best to have a rational plan, rather than an emotional reaction.

Shea Schut - Showmanship

Teach your horse to follow your lead.

Teach your horse to set-up.

- Your horse should learn that when you stop, it should also set-up.

Judie Joba - Hunter/English

A good English prospect is a horse built for endurance, i.e. one with long muscle groups. Since wild horses are typically built for endurance, they often make good English riding prospects.

Ross Graesser - Cowhorse

Unfortunately, I was distracted while Ross gave his presentation. One point of interest he did make while we visited on the side was that to teach a horse to ground tie, a person should dig a small hole, drop the end of the lead rope into it, and pack the dirt over the lead rope. In this way, your horse is literally tied to the ground and will eventually think that every time you drop the lead rope that he is tied to the ground.

Tracy Kleinjan - Trick Training

To teach a horse tricks, you need to give a cue and stimulate the desired response, and then reward the horse. Tracy rewards with a handful of grain. Her horse is being taught to nod yes, shake no, and to bow. She warns that tricks, such as yes and no, can also be bad habits, so a person must think about the consequences prior to teaching tricks.

Don Husted - General Training Tips

Don runs a string of dude horses, and many of those horses are adopted wild ones. When he adopts a horse, he looks at the horse's disposition and place within the herd. He does not like to adopt dominate herd members because dominate animals can be a challenge to gentle. His primary rule for training a wild horse: Don't make an issue out of stuff. Keep a calm, relaxed attitude.

Dave Fisk - Roundpen work

Don't turn training into a contest, but maintain your position of authority.

Jeff Schut - Western Pleasure

Jeff talked about the conformation that makes a good western pleasure prospect. A dip in the neck in front of the withers means that it will be easy for a horse to maintain a low head set. A short back is desirable. A good prospect will have a long, slow stride.

The clinicians imparted more information than I've captured here. These are merely the points I took note of. **Wildhorse Club.calm** plans on sponsoring more of these events in

1: Vet Microbiol. 2002 Nov 6;89(4):311-21.

Construction of a stable non-mucoid deletion mutant of the Streptococcus equi Pinnacle vaccine strain.

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Streptococcus equi causes equine strangles, a purulent lymphadenopathy of the head and neck. An avirulent, non-encapsulated strain (Pinnacle) has been used widely in North America as an intranasal vaccine. The aim of the study was to create a specific mutation of the hyaluronate synthase (hasA) gene in Pinnacle to permanently abolish the production of capsule and provide an easily recognisable genetic marker. An internal fragment of hasA was generated by PCR and cloned into pTW100 (Microscience, UK). An encapsulated revertant of Pinnacle was then transformed with the recombinai plasmid by electroporation and cultured under conditions to promote homologous recombination. Among 90 spectinomycin resistant transformant observed, one non-mucoid (non-encapsulated) spectinomycin resistant colon was detected. The presence of plasmid sequence within the hasA gene was confirmed by the PCR. After six passages in antibiotic-free medium, four non-mucoid spectinomycin sensitive colonies were found. Sequence analysis of one of these clones, designated Pinnacle HasNeg, revealed loss of the 3' end of the hasA and the 5' end of the hasB genes. This deletion mutant shoul serve as a useful candidate to replace Pinnacle since it cannot revert to a mucoid phenotype and can be distinguished genetically from wild type strains.

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1: Infect Immun. 2004 Jun;72(6):3228-36.

Recombinant Streptococcus equi proteins protect mice in challenge experiments and induce immune response in horses.

Flock M, Jacobsson K, Frykberg L, Hirst TR, Franklin A, Guss B, Flock JI.

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Horses that have undergone infection caused by Streptococcus equi subspecies equi (strangles) were found to have significantly increased serum antibody titers against three previously characterized proteins, FNZ (cell surface-bound fibronectin binding protein), SFS (secreted fibronectin binding protein), and EAG (alpha2-macroglobulin, albumin, and immunoglobulin G [IgG] binding protein) from S. equi. To assess the protective efficacy of vaccination with these three proteins, a mouse model of equine strangles was utilized. Parts of the three recombinant proteins were used to immunize mice either subcutaneously or intranasally, prior to nasal challenge with S. equi subsp. equi. The adjuvant used was EtxB, a recombinant form of the B subunit of Escherichia coli heat-labile enterotoxin. It was shown that nasal colonization of S. equi subsp. equi and weight loss due to infection were significantly reduced after vaccination compared with a mock-vaccinated control group. This effect was more pronounced after intranasal vaccination than after subcutaneous vaccination; nearly complete eradication of nasal colonization was obtained after intranasal vaccination ($P < 0.001$). When the same antigens were administered both intranasally and subcutaneously to healthy horses, significant mucosal IgA and serum IgG antibody responses against FNZ and EAG were obtained. The antibody response was enhanced when EtxB was used as an adjuvant. No adverse effects of the antigens or EtxB were observed. Thus, FNZ and EAG in conjunction with EtxB are promising candidates for an efficacious and safe vaccine against strangles.

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